PB82-154980

Perspectives in Chronobiology of Air Pollution

Institut National de la Sante et de la Recherche
Medicale, Paris (France)

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

10 Jan 77

U.S. Department of Commerce
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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

XX-79-015

1. REPORT NO.	2.	3.
EPA-600/J-77-184	JOURNAL ARTICLE	PB82-154980

4. TITLE AND SUBTITLE	5. REPORT DATE
Perspectives In Chronobiology of Air Pollution	December 1981
	6. PERFORMING ORGANIZATION CODE 026080

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Maurice Stupfel, Franz Halberg*, Madeleine Mordelet-Dambrine, and Monique Magnier	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
French National Institute of Health (INSERM) Paris, France *Chronobiology Laboratories University of Minnesota Minneapolis, Minnesota 55455	11. CONTRACT/GRANT NO. Grant R-804512

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Office of Research and Development (RTP,NC) Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Published In: Chronobiologia 4(4): 333-351, 1977

16. ABSTRACT

In a series of experiments, male and female Sprague Dawley rats, kept in light (L) from 06^{00} to 18^{00} alternating with darkness (LD 12:12) inhaled different concentrations of carbon monoxide (50-1,700 ppm) at each of two test times, 12h apart. A decrease in flow of CO_2 ($V\ co_2$) resulting from CO inhalation was greater in the active dark (D) than resting light (L) span. Experimental hypoxic mortality of male and female mice also shows circadian variations, being greater in the D than in the L span. Moreover, a difference of mortality was observed between hypoxic exposures performed at 12^{00} (in LD or DL) and hypoxic exposures performed at 00^{00} (in LD or DL).

Such results await tests of any extent to which they model responses of human beings to air pollution. In human beings any external environmental circadian, circaseptan, and circannual variations in air pollution as such may serve to a variable extent as socio-economic synchronizers of innate rhythms with a corresponding frequency, rather than as solely generators of time patterns in any physiopathologic response to air pollution.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	22
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA-600/J-77-134
Journal Article

reprint from
CHRONOBIOLOGIA Vol. IV - October-December 1977 - Number 4

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PERSPECTIVES IN CHRONOBIOLOGY OF AIR POLLUTION

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Environmental pollution enters more and more as an important factor into considerations of physiopathology, even if a multifactorial etiology of human diseases always includes a genetic source as well. Although much conjecture rather than quantification underlies pertinent statements, it has been presumed that eighty percent of human cancers have an environmental origin. Cigarette smoking is being held responsible for much respiratory incapacitation and many myocardial infarctions. The physiopathological influence of occupational air pollution has been demonstrated in specific exposures: respiratory reactions to inhaled organic materials (e.g. byssinosis), plastics (e.g. vinyl chloride), chemicals (e.g. organic solvents) or other products of industries (e.g. asbestos).

The damages to health by environmental air pollution, as it prevails in big cities, are more subtle to ascertain. However, they have been well established during acute urban air pollution episodes which were characterized by increases of morbidity and mortality in high risk patients (AYRES [1]). The most calamitous of these accidents occurred in the winter in London as well as in certain other big cities. Likewise pollen allergy is a respiratory syndrome related to the seasonal bioperiodicity of blooming plants.

Most air pollutant emissions present a well defined periodicity: circadian (about-every 24 h), circaseptan (about-weekly), circannual (about-seasonal) and possibly an infrannual (pluriannual) rhythmicity. Some organisms already are documented to exhibit changes with one or several of these frequencies, even in the absence of

Key-words: Air pollution; Chronobiology; Circadian rhythm; Circannual rhythm; Circaseptan rhythm; Circatrigintan rhythm.

Supported by the U.S. Public Health Service (5-K06-GM-13981-14) and the U.S. Environmental Protection Agency (R804512-01-0).

Received January 10, 1977.

Chronobiologia *4*, 333, 1977.

known environmental changes with the same frequency. Hence, the question may be raised whether a chronobiological approach could not help by leading to a better understanding of air pollution effects on human health. In any event, it could be worthwhile to investigate whether, and if so when and to what extent, external cycles in air pollutants synchronize and reinforce or when they rather interfere with a spectrum of internal (or endogenous) rhythms so characteristic of human physiology or physiopathology (Fig. 1).

MATERIALS AND METHODS

According to HALBERG and REINBERG [21] biorhythms can be classified by comparison to the period of a day (*dies* = 24-h day) into ultradian, circadian and infradian rhythms, Tab. 1.

Ultradian rhythms

Ultradian rhythms (with a frequency of one cycle in less than 20 h) include the endogenously generated yet environmentally modulated human rhythmicity of heart and respiratory rates and of action potentials revealed by the ECG and EEG, to cite but a few examples. The corresponding frequencies are not known for changes in air pollution but the effect of the latter upon the EEG, ECG and other endogenous periodicities remains of great interest.

Circadian rhythms

Circadian rhythms (with a frequency of one cycle in 20-28 h) are more readily synchronized by environmental cycles than ultradian ones. However, the genetic origin of those circadian changes qualifying as biorhythms, on the basis of tests for persistence and polarity in rate of adjustment following schedule shifts, is no longer seriously questioned.

In the field, the synchronizer can be the light-dark cycle (with a mean 24-h period) associated with the terrestrial rotation (with its mean current sidereal period of 23 h 56 m) and/or social schedules governing the alternance of sleep-wakefulness in human beings. A cycle of about 24 h in air pollution is again a consequence not only of human socioeconomic activity, such as that in industry, automobile traffic, heating and incineration, but also of solar photolysis and within-day meteorologic changes-caloric exchanges in ground atmosphere and in winds.

We have demonstrated in Sprague Dawley and CFE rats, kept under standardized experimental conditions with respect to certain microbial pathology (SPF: specific pathogen free), as well as environmental temperature (20-22° C) relative humidity (50-70%) and lighting (synchronized by a regimen of L 06^{00} to 18^{00}, D 18^{00} to 06^{00}; light intensity: 150 lux), that carbon monoxide is more toxic during the scotofraction (dark span) than during the photofraction (light span). Fig. 2 shows the continuously recorded emission of carbon dioxide (proposed by STUPFEL [52] as an index of gaseous metabolism and activity) of 17 male rats synchronized by alternating L and D. Rats are nocturnally active rodents: the maximum of the % CO_2 concentration in the metabolic chamber corresponds roughly to the maximum of activity and occurs in the scotofraction. When a concentration of 300 ppm carbon monoxide is introduced

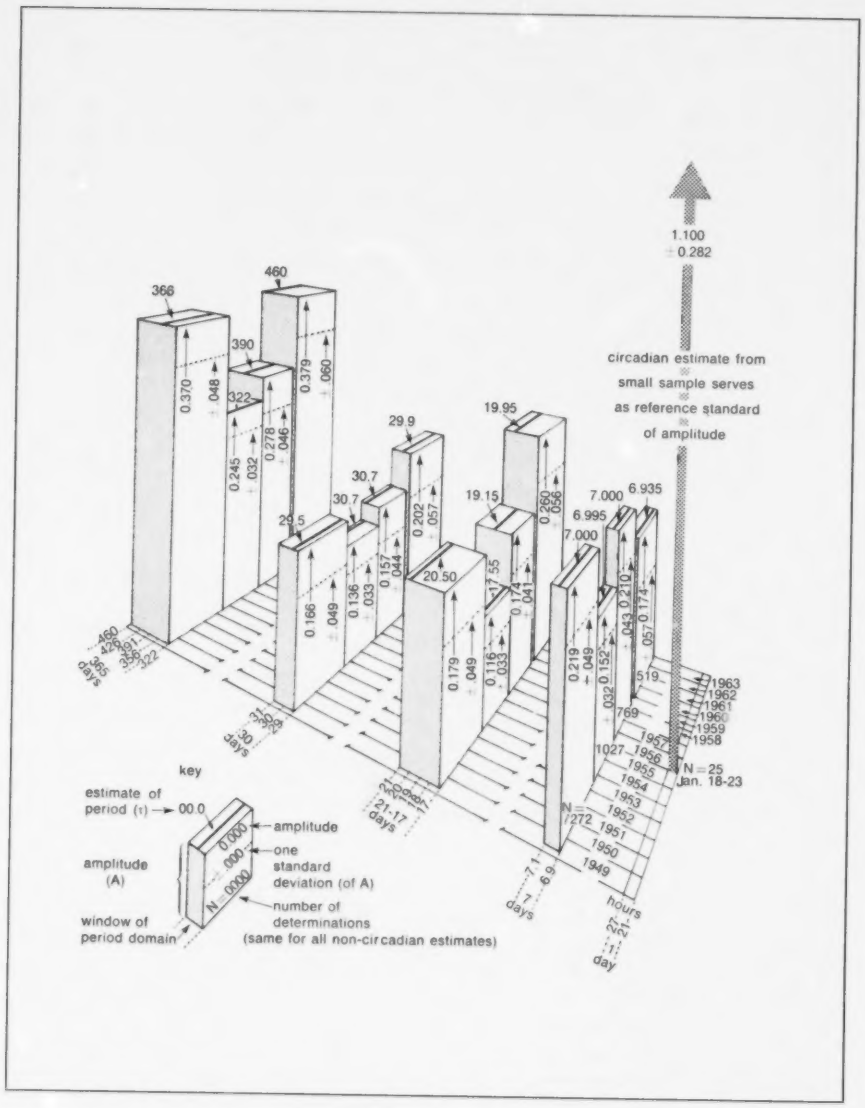


Fig. 1 - Estimates of periods, amplitudes and standard deviations in four period-domains—analyzed by least-squares spectra. Data on human 17-ketosteroid excretion covering more than 15 calendar years, analyzed in four separate sections.

spectral classification of human rhythms

domain *	ultradian	circadian	infradian **
frequency, f (cycles /24 h)	86400		
scales	logarithmic	linear	logarithmic
period, τ (f = 1/τ)	< 20 h	20 h ≤ τ ≤ 28 h	> 28 h
rhythms in health:	electroencephalogram electrocardiogram respiration peristalsis rem. rest-activity	sleep-wakefulness responses to drugs blood constituents urinary variables metabolic processes, generally	menstruation 17-ketosteroid and noreprinephrine excretion
grossly apparent morbidity and mortality rhythms	cf. conventional textbooks of medicine.	• convulsive disorders, intermittent catatonia. morbidity and mortality from certain respiratory, • cardiovascular and other diseases, etc.	

* Domain [named according to frequency (f) by analogy to the physical classification of *ultra*violet or *ultra*sound and *infra*red or *infra*sound, respectively, but delineated according to period (τ), of function approximating rhythm].

** Infradian domain can be subdivided into several regions, as follows: circaseptan (τ∼7 d); circavigintan (τ∼20 d); circatrigintan (τ∼ 30 d); and circannual (τ∼1 yr). Regions in the ultradian and circadian domains have not as yet been delineated, except for the 'specific range' of the EEG (cycles/sec): delta (<1-3.5); theta (3.6-7); alpha (7.1-12); beta (12.1-30).
Several variables examined thus far exhibit statistically significant components in all three spectral domains, as does the EEG. 17-ketosteroid and norepinephrine rhythms with circadian frequency also are quite prominent.

Tab. 1 - Spectral classification of human rhythms.

during the photofraction (either between 13^{00} and 17^{00} or between 11^{00} and 13^{00}) no apparent effect is induced on the curve of the CO_2 emitted by the rats; but when this same concentration of 300 ppm CO is introduced during the scotofraction (between 23^{30} and 00^{30}) a sharp decrease of the CO_2 emitted by the rats is noted. This decrease of $\dot{V}_{CO_2}$ corresponds to a diminution of oxygen consumption and of general body activity and hence to a toxic effect. Twenty-one experiments were performed on the same group of aging male rats between 16 and 19 months of age (consisting initially of 22 and finally of 16 animals). Nine different concentrations of carbon monoxide (50, 125, 250, 300, 400, 450, 500, 600 and 650 ppm) were delivered to them either during the photofraction or during the scotofraction. In all these experiments, a given concentration of carbon monoxide lowered the $\dot{V}_{CO_2}$ more in the scotofraction than in the photofraction (STUPFEL et al. [55]).

We report herein, for the first time, the results of 18 experiments made with the previously described techniques on groups of 28-41 male or female rats which inhaled 5 different concentrations of carbon monoxide (50, 300, 480, 1000 and 1700 ppm). Animals between the ages of 4 and 14 months were exposed at two

circadian times 12 h apart: either during the middle of the daily photofraction (between 10^{30} and 13^{30}) or during the middle of the daily scotofraction (between 22^{30} and 01^{30}). The percentage of variation in $\dot{V}_{CO_2}$ at 1, 2 or 3 h after the beginning of the carbon monoxide inhalation reflects the effect of CO on the rats' gaseous metabolism and motor activity. Fig. 3 shows the variations of $\dot{V}_{CO_2}$ ($\Delta\dot{V}_{CO_2}$ %) on ordinates during the circadian photofraction (dotted lines) and the circadian scotofraction (solid lines) for different concentrations of carbon monoxide on abscissas. It must be noted that the effects are biased by uncontrolled differences in age. However, as arguments against exclusive age effects one might indicate that the lowest dose was given at one time to the youngest and at another time to the oldest animals. Nonetheless, the change in $\dot{V}_{CO_2}$ at all times of exposure as a function of CO dose was in the anticipated direction. Moreover, one can compare, on pooled data on animals of similar age, the effect of the fixed dose (300 ppm) given at different times, to find that this dose acted differently. All in all, these arguments suggest that we are not dealing with mere age effects. However, to draw more rigorous conclusions, animals of the same age will have to be examined at all exposure times in any future studies.

Tab. 2 summarizes the effect of CO inhalation on $\dot{V}_{CO_2}$ of male and female rats, during the photofraction and scotofraction, respectively. The result of a linear regression analysis shows a decrease in $\dot{V}_{CO_2}$ when the concentration of inhaled CO increases. According to Tab. 2, the statistical significance of any linear dependence

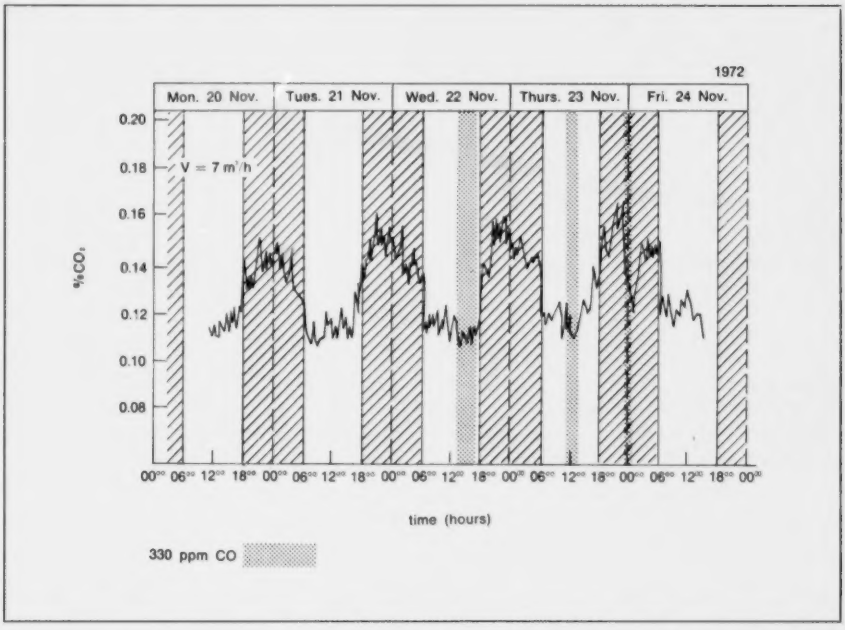


Fig. 2 - A group of seventeen Sprague Dawley CFE male rats synchronized by light (06^{00}-18^{00}) and darkness (18^{00}-06^{00}) as shown by circadian rhythm of CO_2 emission (continuously recorded). Concentration of 300 ppm of carbon monoxide is given at different times of the light and dark spans (shaded area). Only the inhalation at 00^{00} provokes a diminution of the carbon dioxide emission.

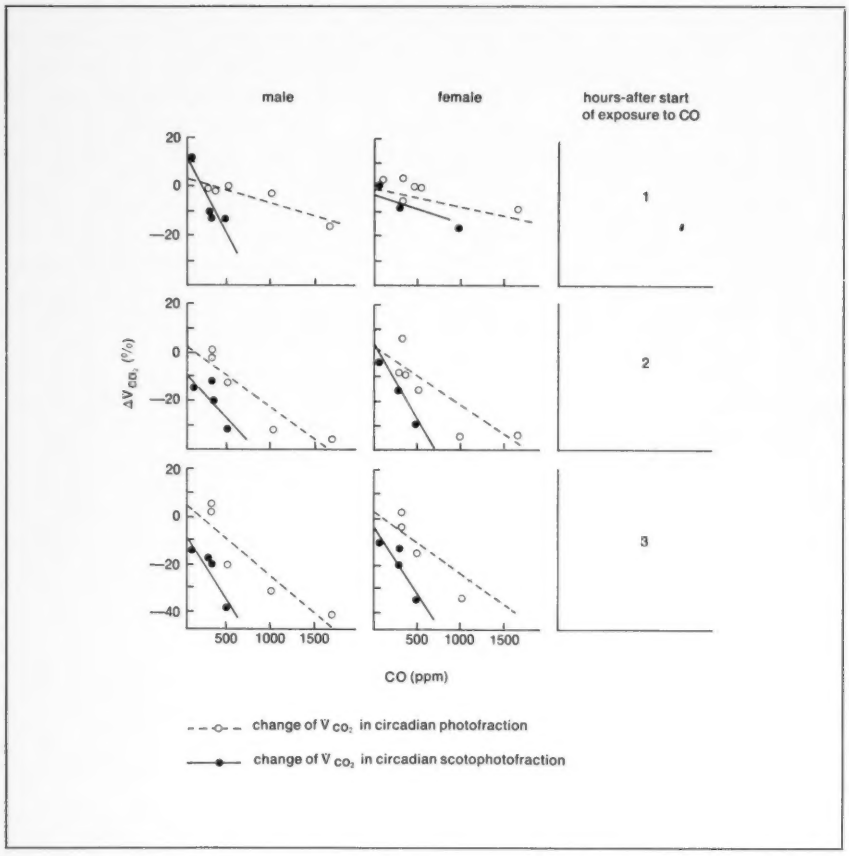


Fig. 3 - Comparison of paired slopes of CO_2 emission in response to graded exposure to CO (all data).

of $\dot{V}_{CO_2}$ upon CO concentration appears to depend on the time of exposure to CO (photofraction or scotofraction) and is more apparent in males than in females. This is shown in the last column of Tab. 2 by the p-value related to an F-test for a zero slope of the straight line. More specifically, if we denote CO ppm as x_i and $\dot{V}_{CO_2}$ as y_i, $\bar{x}$ and $\bar{y}$ being the corresponding means, and $y_i = \beta_o + \beta_1 x_i$, F-ratios:

$$F = \beta_1 \left\{ \sum_{i=1}^{n} (x_i - \bar{x})\ (y_i - \bar{y}) \right\} / \left[\sum_{i=1}^{n} (y_i - \hat{y}_i)^2 / (n-2) \right] \qquad (1)$$

(with $\hat{y}_i$ being the estimate of y_i) can be constructed to test the significance of the slopes and then be compared to the F-value for the F-distribution with (1, n-2) degrees of freedom (DRAPER and SMITH [10]).

A statistical comparison of the slopes obtained during the photofraction and scotofraction, respectively, shows that a significant difference was found in only one

condition	no.	intercept	slope**	(SE)	F	(D.F.)	p
M (light) 1h	5	4.11	—0.10	(0.22)	20.143	(1,3)	<0.025
(light) 2h	5	3.30	—2.48	(0.51)	23.808	(1,3)	<0.025
(light) 3h	5	5.22	—2.88	(0.78)	13.564	(1,3)	<0.05
M (dark) 1h	4	12.14	—6.24	(2.06)	9.200	(1,2)	>0.05
(dark) 2h	4	—8.19	—3.66	(2.26)	2.621	(1,2)	>0.10
(dark) 3h	4	—7.53	—4.99	(1.96)	6.494	(1,2)	>0.10
F (light) 1h	5	—0.23	—0.67	(0.61)	1.202	(1,3)	>0.25
(light) 2h	5	0.24	—2.20	(0.89)	6.08	(1,3)	>0.05
(light) 3h	5	1.01	—2.30	(0.68)	11.320	(1,3)	<0.05
F (dark) 1h	4	—3.13	—0.96	(4.04)	.056	(1,2)	>0.25
(dark) 2h	4	1.21	—5.38	(1.70)	10.004	(1,2)	>0.05
(dark) 3h	4	—5.21	—4.97	(2.10)	5.612	(1,2)	>0.10
(M + F)							
(light) 1h	10	1.94	—0.82	(0.29)	7.845	(1,8)	<0.025
(light) 2h	10	1.77	—2.34	(0.45)	27.276	(1,8)	<0.001
(light) 3h	10	3.11	—2.59	(0.46)	31.478	(1,8)	<0.001
(M + F)							
(dark) 1h	8	4.50	—3.60	(2.14)	2.824	(1,6)	>0.10
(dark) 2h	8	—3.49	—4.52	(1.35)	11.195	(1,6)	<0.025
(dark) 3h	8	—6.37	—4.98	(1.21)	16.825	(1,6)	<0.01

* Dose-response slopes, separately at each of three times after start of inhalation (1, 2 or 3 h, as shown in Fig. 3). M = males; F = females; M + F = males and females.

** Slope on transformed scale of CO ppm. Actual slope should be multiplied by 10^{-2}.

Tab. 2 - Summary of CO_2 emission studies in Fig. 3 *.

of six comparisons, a point more readily apparent in Fig. 3. Paired slopes are shown in Fig. 3 and Fig. 4, straight lines being fitted in each case by the least-squares method to % changes in $\dot{V}_{CO_2}$, separately for data obtained from male and female rats at different times from start of exposure in light or darkness.

Table 2 as well as Figs 3 and 4 show that the inhalation of 300 ppm of CO in the photofraction (10^{30} to 13^{30}) had no apparent effect on the $\dot{V}_{CO_2}$ of the males and little negative action (—6.86%, —8.82%, —4.90%) on the $\dot{V}_{CO_2}$ of only one of the two groups of 41 females studied (Tab. 2). The inhalation of 300 ppm CO during the scotofraction (22^{30} to 01^{30}) decreased $\dot{V}_{CO_2}$ in every group from both sexes. Moreover, $\dot{V}_{CO_2}$ diminutions were obtained in males and females inhaling 50 ppm CO during the scotofraction. Furthermore, breathing 480 ppm CO during the scotofraction decreased the $\dot{V}_{CO_2}$ about as much as breathing 1700 ppm CO during the photofraction *.

* In order to find out whether the slopes for the response (change in CO_2 emission) of animals kept on LD 12:12 regimen exposed to varying concentrations of CO in the daily light span are the same as those of animals exposed during the daily darkness, a sign test may be used. This test applied to all of the Fig. 4 comparisons yields a p-value to 0.0156. Thus, one can reject the null hypothesis of no difference in slope as a function of exposure during the photofraction or the scotofraction.

We obtained similar results also in male and female OF_1, SPF mice, kept again under conditions controlled for environmental temperature and humidity, in LD 12:12 (L = 06^{00} - 18^{00}), and inhaling during 20 min a mixture of 5.5% oxygen in 94.5% nitrogen (LD_{50} concentration). This nitrogen hypoxia could be compared with the hypoxic effect of 0.20% carbon monoxide in air. Exposure to inhalation of this 5.5% O_2 mixture was studied during the same day at 12^{00} and at 00^{00}, each time on male and female mice, previously standardized for 1 month either in light from 06^{00} to 18^{00} (LD), or in light from 18^{00} to 06^{00} (DL).

Table 3 shows the cumulated mortalities observed at the end of the 20-min exposure to the 5.5% O_2 in 26 two-timepoint studies, i.e. in exposure sessions on a total of 1,309 males and 1,305 females of similar age, 30-40 days of age at the time of exposure. As can be seen from the first two lines of the mortality data of Tab. 3 and from Tab. 4, the hypoxic mortality is higher during the scotofraction at 12^{00} in DL and at 00^{00} in LD, than during the photofraction at 12^{00} in LD and at 00^{00} in DL.

In these nocturnal rodents the scotofraction corresponds to the span of heightened gross body activity and the photofraction is largely a rest span. In the last three lines of Tab. 3, the mortality data obtained during LD (06^{00} - 18^{00}) and DL (18^{00} - 06^{00}) regimens have been pooled separately for the results of hypoxic exposure performed at 12^{00} (in the two columns on the left in Tab. 3) or for the hypoxic exposure performed at 00^{00} (in the two columns on the right of Tab. 3). Thus one obtains the overall mortality from exposure at 12^{00} or at 00^{00}, independently of any lighting regimen. When the hypoxic exposure is performed at 12^{00} in males the mortality is higher (58%) than when the exposure to hypoxia occurs at 00^{00} (50%). In females the hypoxic mortality is also higher at 12^{00} than at 00^{00} (44 versus 37%). A circadian within-day difference, in spotchecks carried out 12 h apart, seems to characterize

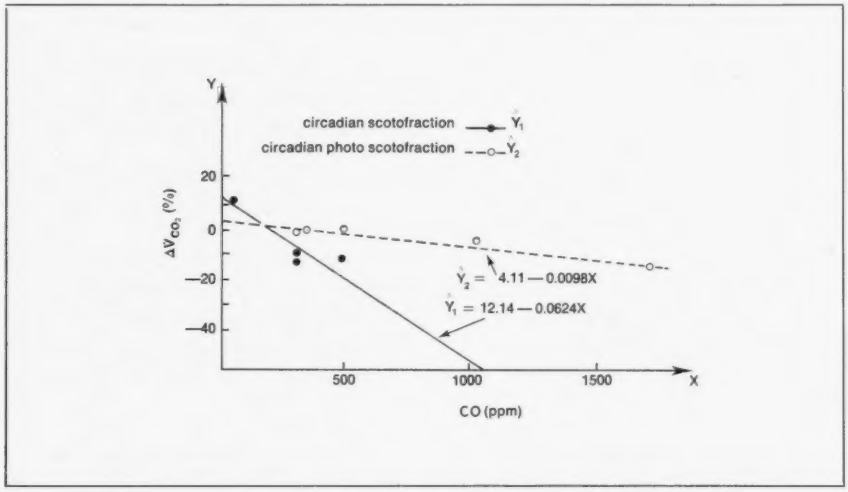


Fig. 4 - Statistically significant difference from comparison of paired slopes for males at 1 h after start of exposure to CO ($p<.025$).

time of hypoxia	12^{00}		00^{00}		12^{00}		00^{00}	
sex	males				females			
light	LD	DL	DL	LD	LD	DL	DL	LD
dead mice/ total mice	138/326	240/324	128/326	200/333	110/331	179/333	110/324	131/324
%	42.33	74.07	39.26	60.06	33.23	53.75	33.95	40.43
dead mice/ total mice	$\frac{138+240}{326+324}=\frac{378}{650}$		$\frac{128+200}{326+333}=\frac{328}{659}$		$\frac{110+179}{331+333}=\frac{289}{657}$		$\frac{110+131}{324+324}=\frac{241}{648}$	
%	58.15		49.77		43.99		37.19	

LD = light 06^{00}-18^{00} and dark 18^{00}-06^{00}
DL = dark 18^{00}-06^{00} and light 06^{00}-18^{00}

Tab. 3 - Mortality of male and female mice inhaling 5.5% O_2 at 2 different times of the day and in different lighting regimens.

hypoxic mortality, independently of the previously noted difference between the photo and the scotofractions.

If they could be corroborated with additional samples, the Tab. 3 results would be most interesting. Local effects upon rhythms for a long time have been claimed to occur but have been very difficult to demonstrate with statistical significance, as shown in Tab. 3, unfortunately only with a two timepoint approach. Moreover, the difference found was not predicted. Hence, only tentative inferences are drawn from the results in Tab. 3. With such qualifying restriction, one has to consider that some local factor, uncontrolled or subtle, may have contributed to the results.

Animal experimentation reported in the literature yields many examples of circadian differences in susceptibility to potentially toxic agents (STUPFEL [53,54]). If and only if they are standardized with a 24 h periodic environmental regimen (such as LD 12:12) nocturnal rodents exhibit a higher sensitivity to fluothane or halothane during the dark (activity) span (MATTHEWS et al. [30]; MUNSON et al. [33]) than during the light (rest) span.

The same finding applies to a variety of other agents affecting the central nervous system, such as ethanol (HAUS and HALBERG [23]), librium (MARTE and HALBERG [29]) and even noise (HALBERG et al. [16,19]). What is important, however, is the demonstration that such rhythms in susceptibility persist in the absence of known environmental synchronizers e.g. in continuous darkness or continuous illumination (HALBERG [12]; SCHEVING et al. [42]). Even 7,12 dimethylbenz(a)anthracene is more effective in inducing papillomata of the skin in mice when it is applied at 22^{00}, as compared to application at 10^{00} (MOTTRAM [32]), Fig. 5, and such susceptibility rhythms to carcinogens gain in importance as we recognize the role played by environmental factors in carcinogenesis (HALBERG et al. [18]).

Human data on circadian changes in susceptibility to potentially harmful challenge by constituents of the air for the moment are relatively scarce (KERR [24]; SMOLENSKY

et al.[46]). Circadian differences in sensitivity to acute hypoxia have been reported in human beings (SCHWARTZ [44]) and could be likened to our findings in mice, by taking into account the fact that human beings are diurnally active mammals while mice are nocturnally active. Circadian reactivity rhythms of human skin to house dust and histamine have been experimentally observed (REINBERG et al. [38,39]). Results of many endocrinological studies enable us to anticipate human circadian variations in susceptibility to different types of potentially harmful challenge (HALBERG [13,14]; REINBERG and HALBERG [38]; STUPFEL [53,54]).

Infradian rhythms

a. *Circaseptan rhythms.* Circaseptan rhythms (with a period of about 7 days) are societal with respect to the well documented air pollution by automobile traffic (BULLOCK and LEWIS [4]; U.S. Department of Commerce [8]). The interruption of occupational exposure to organic air pollutants during weekends has created in the cotton, flax and hemp industries a definitely uneven occurrence of pathology along the scale of a week. The so-called *Monday syndrome* (cough, dyspnea, diminution of forced expiratory volume and altered serum immunological reactions) is a case in point (BOUHUYS [3]; BARBERO and FLORES [2]; MOLINA [31]; KILBURN [25]). It is also well known that hospital admissions are higher during weekdays than during weekends (STERLING et al. [51]). Most interesting are circaseptan variations, of mammalian response to kidney allografting (RATTE et al. [35,36]) in the context of broader presumably

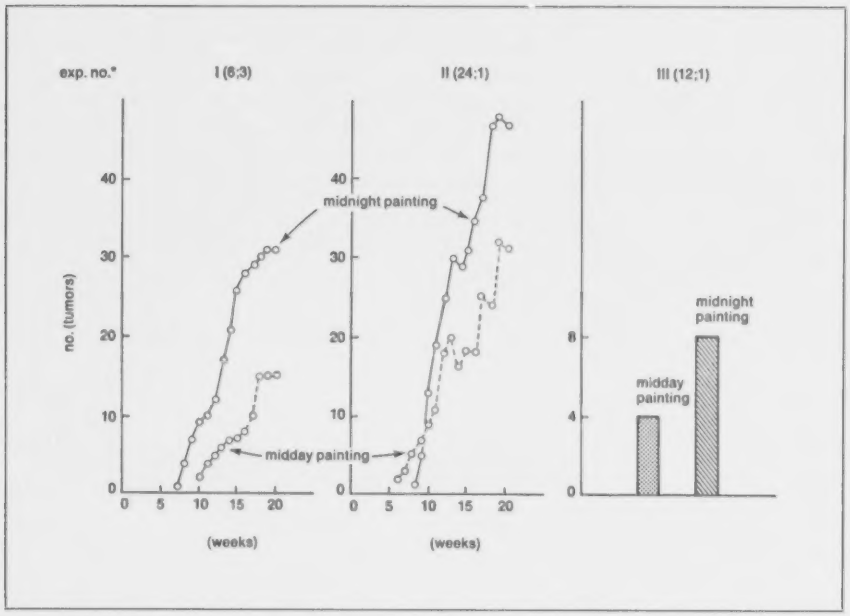
sex investigated	males		
comparisons made	12^{00} (LD) v** 00^{00} (LD)	12^{00} (DL) v 00^{00} (DL)	12^{00} (LD+DL) v 00^{00} (LD+DL)
χ^2 (computed)	20.021	78.756	8.918
p (2-sided)	<0.001	<0.001	<0.01

sex investigated	females		
comparisons made	12^{00} (LD) v 00^{00} (LD)	12^{00} (DL) v 00^{00} (DL)	12^{00} (LD+DL) v 00^{00} (LD+DL)
χ^2 (computed)	3.346	25.341	5.969
p (2-sided)	>0.1	<0.001	<0.05

* on Tab. 3 data

** versus

Tab. 4 - Chi-square test results *.



* No. of mice and of applications of carcinogen, in this order, in parentheses; croton oil applied after the carcinogen in I and II (60 appl.) and before (5 appl.) in III.

Fig. 5 - Papillomata of the skin induced in mice by painting with 7,12 dimethylbenz (a) anthracene.

endogenous circaseptan bioperiodicity (HALBERG et al.[20]). HALBERG et al.[17] have documented endogenous human circaseptan periodicity of urinary 17-ketosteroids by the demonstration of a free-running rhythm in a Danish physician-scientist who collected and analyzed his urines over a decade and one-half, Fig. 6.

b. *Circatrigintan (menstrual) rhythms.* Such rhythms characterize vital capacity, alveolar pCO_2 and other variables as can be seen from Fig. 7. They will contribute a source of variability to the study of rhythms with higher and lower frequency that may be more readily or more clearly synchronized by environmental cycles including air pollution.

c. *Circannual rhythms.* A cycle with a mean period of 365.25 days results from the circannual rotation of the earth around the sun with seasonal changes in temperature, light and also vegetation (food) as potentially synchronizing consequences *. The effects of such geophysical factors on human health constitute partly the subject

* Fig. 8 demonstrates a one-year synchronized circannual rhythm in urine volume and, in the same urine, a rhythm with a period seemingly different from a precise one-year span, for 17-ketosteroid excretion. Additional and longer series of data are needed for a more vigorous documentation of a deviation of the circannual period from the precise one-year span in human beings and thus for an indirect documentation of innate circannual rhythms. Other studies carried out with Dr. Bangalore Sundararaj in the Department of Zoology, Delhi University, India, under conditions of constant darkness, have provided an example of an innate circannual rhythm in ovarian recrudescence of the catfish (Int. J. Chronobiol. *1*, 362, 1973).

of biometeorology (DERUDDER [9]; PETERSON [34]); these effects are difficult to investigate scientifically since they necessitate measurements or observations during spans of several years and, what is more difficult to implement, studies under controlled conditions for such durations are expensive. Moreover, during time spans of years one may find in human beings the superimposed effect of growth or aging. In experimentation on rodents with short lifespans (mice, rats: 2-3 years of life) the confounding of effects of age and circannual rhythm is particularly noteworthy.

In any event, chronoepidemiology has begun to quantify the importance of circannual factors in many diseases. Influenza and respiratory diseases predominate

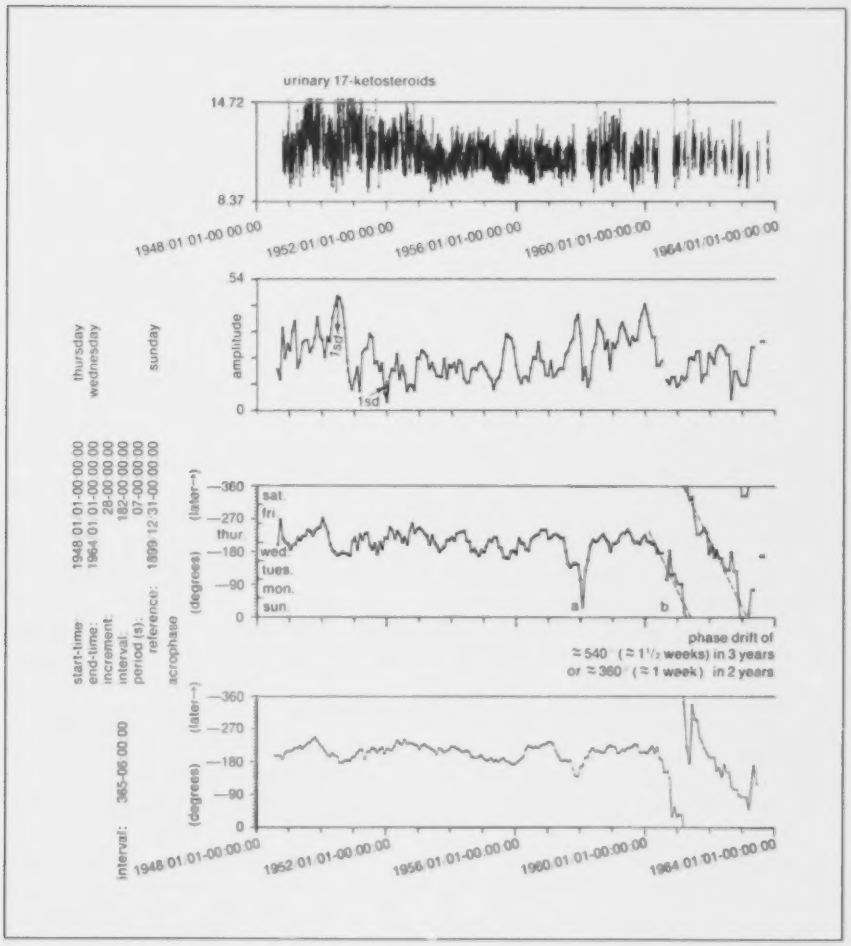
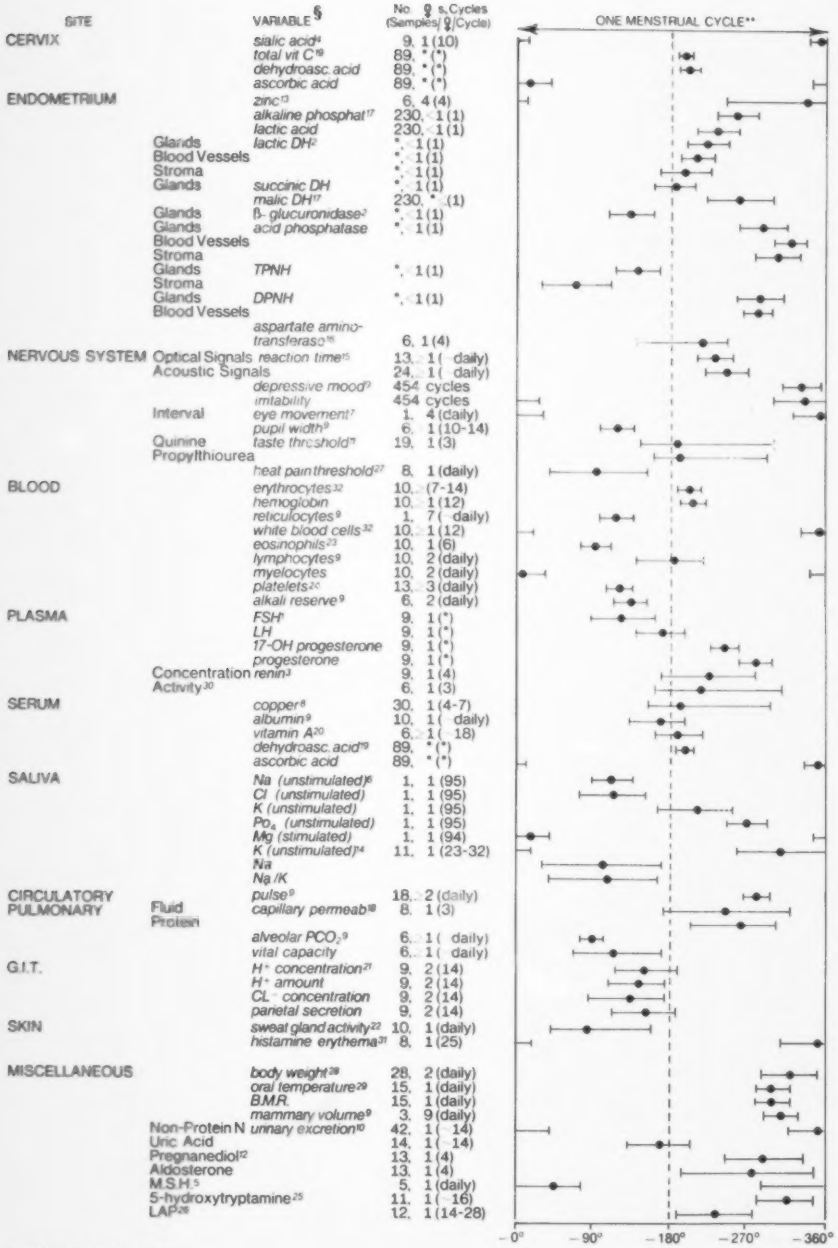


Fig. 6 - 17-ketosteroid excretion, plotted as a function of time, on top, evaluated below by a temporal amplitude diagram and by two phase diagrams. Precise 7 - day period fitted, with increments of 28 days, to consecutive overlapping intervals of 182 or 365 days.


SITE
VARIABLE §
No. ♀ s./Cycles (Samples/♀/Cycle)
ONE MENSTRUAL CYCLE**
CERVIX
sialic acid[4] 9. 1 (10)
total vit C[19] 89. * (*)
dehydroasc. acid 89. * (*)
ascorbic acid 89. * (*)
ENDOMETRIUM
zinc[13] 6. 4 (4)
alkaline phosphat[17] 230. 1 (1)
lactic acid 230. 1 (1)
Glands lactic DH[2] *. 1 (1)
Blood Vessels *. 1 (1)
Stroma *. 1 (1)
Glands succinic DH *. 1 (1)
malic DH[17] 230. *. (1)
Glands ß-glucuronidase[2] *. 1 (1)
Glands acid phosphatase *. 1 (1)
Blood Vessels
Stroma
Glands TPNH *. 1 (1)
Stroma
Glands DPNH *. 1 (1)
Blood Vessels
aspartate amino-transferase[16] 6. 1 (4)
NERVOUS SYSTEM Optical Signals reaction time[15] 13. 1 (daily)
Acoustic Signals 24. 1 (daily)
depressive mood[7] 454 cycles
irritability 454 cycles
Interval eye movement[7] 1. 4 (daily)
pupil width[9] 6. 1 (10-14)
Quinine taste threshold[7] 19. 1 (3)
Propylthiourea
heat pain threshold[27] 8. 1 (daily)
BLOOD
erythrocytes[32] 10. (7-14)
hemoglobin 10. 1 (12)
reticulocytes[9] 1. 7 (daily)
white blood cells[32] 10. 1 (12)
eosinophils[23] 10. 1 (6)
lymphocytes[9] 10. 2 (daily)
myelocytes 10. 2 (daily)
platelets[22] 13. 3 (daily)
alkali reserve[9] 6. 2 (daily)
PLASMA
FSH[1] 9. 1 (*)
LH 9. 1 (*)
17-OH progesterone 9. 1 (*)
progesterone 9. 1 (*)
Concentration renin[3] 9. 1 (4)
Activity[30] 6. 1 (3)
SERUM
copper[6] 30. 1 (4-7)
albumin[9] 10. 1 (daily)
vitamin A[20] 6. 1 (18)
dehydroasc. acid[19] 89. * (*)
ascorbic acid 89. * (*)
SALIVA
Na (unstimulated)[6] 1. 1 (95)
Cl (unstimulated) 1. 1 (95)
K (unstimulated) 1. 1 (95)
Po_4 (unstimulated) 1. 1 (95)
Mg (stimulated) 1. 1 (94)
K (unstimulated)[24] 11. 1 (23-32)
Na
Na /K
CIRCULATORY pulse[9] 18. 2 (daily)
PULMONARY Fluid capillary permeab[18] 8. 1 (3)
Protein
alveolar PCO_2[9] 6. 1 (daily)
vital capacity 6. 1 (daily)
G.I.T.
H^+ concentration[21] 9. 2 (14)
H^+ amount 9. 2 (14)
CL^- concentration 9. 2 (14)
parietal secretion 9. 2 (14)
SKIN
sweat gland activity[22] 10. 1 (daily)
histamine erythema[31] 8. 1 (25)
MISCELLANEOUS
body weight[28] 28. 2 (daily)
oral temperature[29] 15. 1 (daily)
B.M.R. 15. 1 (daily)
mammary volume[9] 3. 9 (daily)
Non-Protein N urinary excretion[10] 42. 1 (14)
Uric Acid 14. 1 (14)
Pregnanediol[12] 13. 1 (4)
Aldosterone 13. 1 (4)
M.S.H.[5] 5. 1 (daily)
5-hydroxytryptamine[25] 11. 1 (16)
LAP[26] 12. 1 (14-28)
– 0°
– 90°
– 180°
– 270°
– 360°

* information not given

** internal acrophase. Φ reference: 00° ° on day 1 of menses.
Units depend on period (τ). E.g., if τ = 30 days; 30 days = 360°; 1 day = 12°.

95% confidential interval of Φ

§ CONTRIBUTORS: ABRAHAM[1], BOUTSELIS[2], BROWN[3], CARLBORG[4], DAHLBERG[5], DAWES[6], DE LEE[7], DOKUMOV[8], DORING[9], ERIKSON[10], GRANVILLE[11], GRAY[12], HAGENFELDT[13], HATZIOTIS[14], HILDEBRANDT[15], HOCHMAN[16], HUGES[17], JONES[18], KOFOED[19], LAURENCE[20], MACDONALD[21], MACKINNON[22], PATHAK[23], POHLE[24], POKORNY[25], POLISHUK[26], PROCACCI[27], ROBINSON[28], RUBINSTEIN[29], SKINNER[30], SMOLENSKY[31], VARTIANEN[32].

Fig. 7 - Female circatrigintan (menstrual) system.

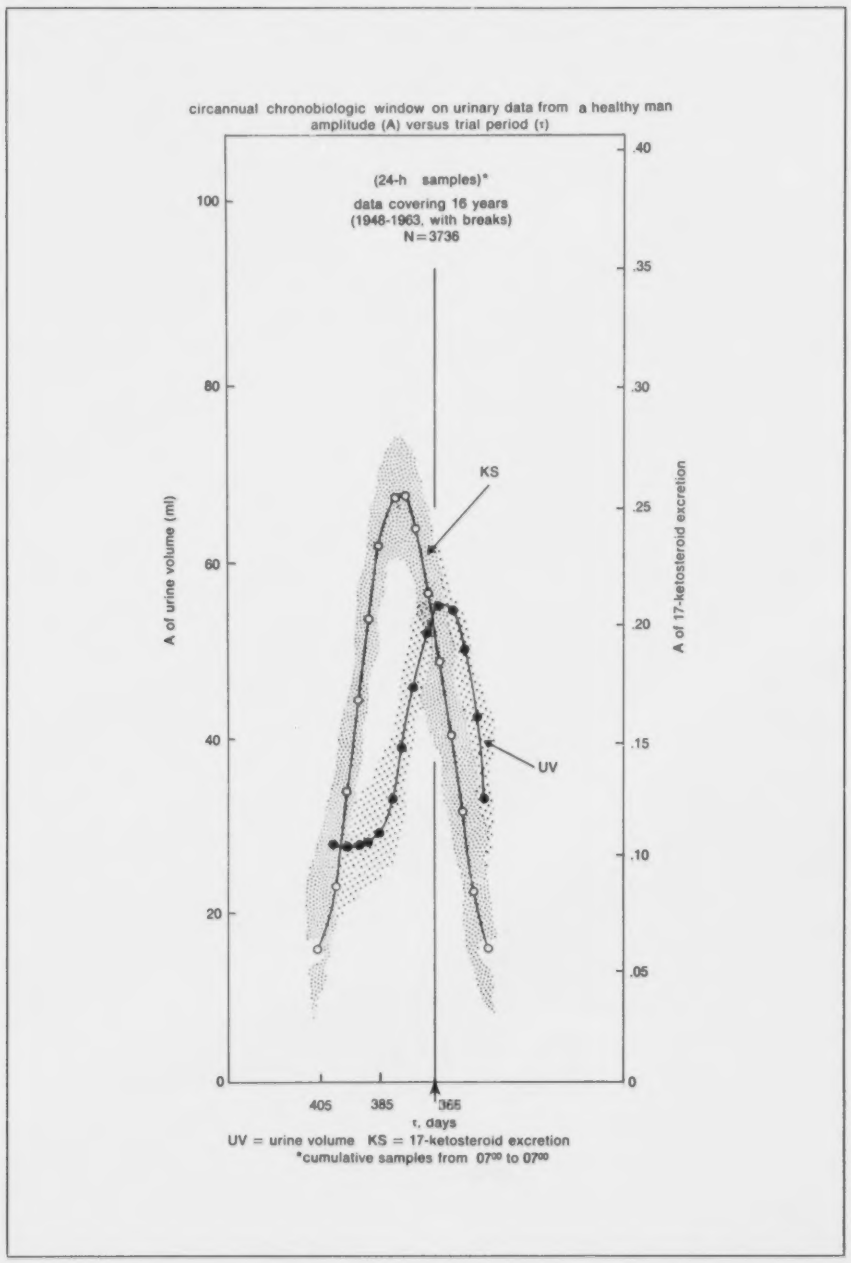


Fig. 8 - Circannual rhythm in 17-ketosteroid excretion; analysis suggests (but does not prove) the operation of a desynchronized about-yearly component in urinary 17-ketosteroids, with a one-year synchronized component in volume excretion.

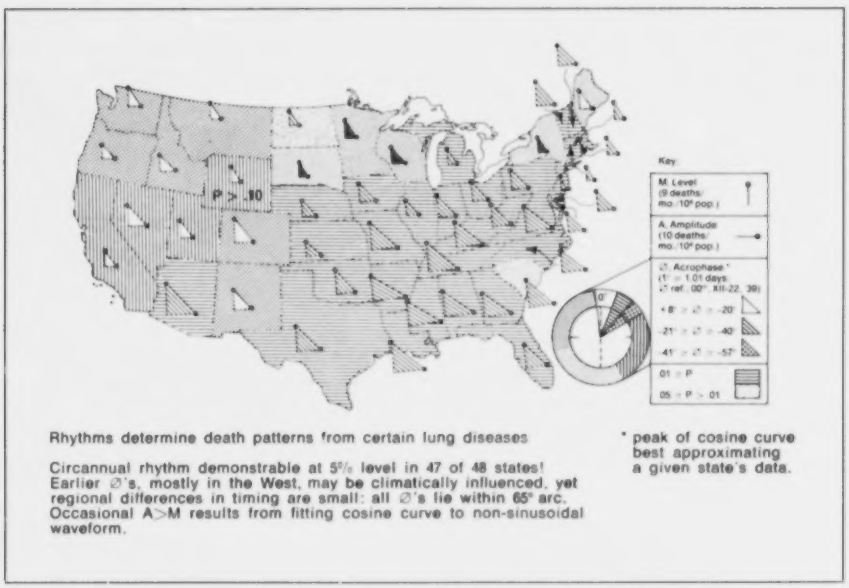

P > .10
Key:
M, Level
(9 deaths/
mo. /10⁶ pop.)
A, Amplitude
(10 deaths/
mo. /10⁶ pop.)
∅, Acrophase *
(1° = 1.01 days
∅ ref. 00°, XII-22, 39)
+8° ≥ ∅ ≥ -20°
-21° ≥ ∅ ≥ -40°
-41° ≥ ∅ ≥ -57°
.01 ≥ P
.05 ≥ P > .01
Rhythms determine death patterns from certain lung diseases
Circannual rhythm demonstrable at 5% level in 47 of 48 states! Earlier ∅'s, mostly in the West, may be climatically influenced, yet regional differences in timing are small: all ∅'s lie within 65° arc. Occasional A>M results from fitting cosine curve to non-sinusoidal waveform.
* peak of cosine curve best approximating a given state's data.

Fig. 9 - 'Months of changing risk' for death from pneumonia and influenza summarized by the fit of a 365.25 - day cosine curve to 1940 - human mortality by state in USA.

in the winter, Fig. 9. Hibernal meteorological aggressions combine with the assault of SO_2, NOx and particles and bacterial as well as viral infections. Other circannual variations of vegetal air pollutants can result in respiratory allergic (clinical and immunological) crises following pollen calendars (WOODHOUSE [56]; SOLOMON [47]; CHARPIN and SURYNIACH [5]; REISMAN et al. [40]).

d. *Infrannual variations.* Variations with a frequency lower than one cycle/year may relate to factors as diverse as socioeconomical or political time trends that contribute air pollution. In considering an 'about 11-year' cycle in sunspots and its variability in this connection, rigorous analyses are needed on data of a kind yet more demanding, both in terms of duration requirements and of difficulties in terms of control than are data for the study of circannual rhythms.

DISCUSSION

The increase in the heating of low atmospheric layers, consequent to the growth of a city, can change progressively the city's microclimate (LANDSBERG [26]), modifying emission, diffusion and removal of air pollution. The increasing number of cars, the development of new sources of energy (as a result of the fuel crisis) and governmental planning in several countries modify both the components and the amount of air pollution.

The multiple facets of these temporal trends should be taken into account in long-term chronoepidemiologic surveys of air pollution. Activities in this field

(COHEN et al.[7]; FINKLEA et al.[11]; HAMMER et al.[22]; LÜBCKE et al.[27]; MANSOURIAN et al.[28]; SCHNEIDER and COSTILOE[43]; STEBBINGS[48]; STEBBINGS et al.[50]; STEBBINGS et al.[51]) await the application of appropriate methods for this purpose, with a view also of chronobiologic facts.

The major aim of this review is to point out first that pollutants in our environment are now documented as exhibiting several frequencies. Pertinent literature references continue to accumulate. Against this background, it is important to point out, next, that the organism itself also is documented to exhibit a number of frequencies or rhythms. By the criterion of the number of cycles/unit time, physiologic rhythms may correspond in frequency, if not casually, to some of the cycles of environmental pollutants. It is hence critical to recognize that our spectrum of innate frequencies need not be the mere result of nurture. One or the other genetically determined component in a rhythm spectrum can be synchronized by one or more environmental factors and can also be changed by the environment in its other characteristics, such as the mesor. An exacerbation of environmental conditions may well be predicted and perhaps ill effects prevented by action based on prior information on the kinds and the characteristics of the frequencies operating in the net-works of interacting nature and nurture.

The overview of the field as a whole remains beyond the scope of this review which attempted only to provide suggestions for further study, with emphasis on the need for replacing single sample potshots by the study of rhythms and thus for developing the necessary methodology to separate any local potentially harmful factors from built-in (genetic) factors by the very characteristics of the periodicities on hand.

CONCLUSIONS

A chronobiological approach views more accurately, and holds promise to clarify, interactions of environmental factors in the production of air pollution, and the physiopathological ways of human reaction to pollution. A classification of the frequencies involved is here endeavored; it indicates that meteorologic and socioeconomic variables modulate both the environmental impact and the ways human beings isolate themselves more and more from the circadian and circannual variations in the temperature, light and food aspects of their environment. Instead, human beings become more and more liable to a socioeconomic environment which also has its circadian, circaseptan and circannual temporal aspects, to cite but a few examples.

When considering the merits or demerits of air quality standards and other means of environmental protection both short-term and long-term periodicities must be taken into account by the choice of an appropriate statistical and physiologic methodology. One should consider the impact of any environmental periodicity in the context of information on the response of the organisms', the cities' and the populations' own spectral structure manifested as a network of rhythms.

SUMMARY

In a series of experiments, male and female Sprague Dawley rats, kept in light (L) from 06^{00} to 18^{00} alternating with darkness (LD 12:12) inhaled different concentrations of carbon monoxide (50-1,700 ppm) at each of two test times, 12 h apart. A decrease in flow of CO_2

($\dot{V}_{CO_2}$) resulting from CO inhalation was greater in the active dark (D) than resting light (L) span. Experimental hypoxic mortality of male and female mice also shows circadian variations, being greater in the D than in the L span. Moreover, a difference of mortality was observed between hypoxic exposures performed at 12^{00} (in LD or DL) and hypoxic exposures performed at 00^{00} (in LD or DL).

Such results await tests of any extent to which they model responses of human beings to air pollution. In human beings any external environmental circadian, circaseptan and circannual variations in air pollution as such may serve to a variable extent as socioeconomic *synchronizers* of innate rhythms with a corresponding frequency, rather than as solely *generators* of time patterns in any physiopathologic response to air pollution.

ACKNOWLEDGEMENTS

Germaine Cornelissen and Jung-Keun Lee, both of the Chronobiology Laboratories, University of Minnesota, provided invaluable help with data analyses. Walter Nelson and William Lubanovic of the same laboratories commented helpfully.

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